

AWARD NUMBER: W81XWH-15-1-0412  
AZ140150

TITLE: Measuring Glial Metabolism in Repetitive Brain Trauma & Alzheimer's Disease

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REPORT DATE: September 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE September 2017		2. REPORT TYPE Annual		3. DATES COVERED 15Aug2016-14Aug2017	
4. TITLE AND SUBTITLE Measuring Glial Metabolism in Repetitive Brain Trauma and  Alzheimer's Disease				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER WX81XWH-15-1-0412	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)  Alexander Lin, PhD  E-Mail: aplin@bwh.harvard.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  Brigham and Women's Hospital 75 Francis Street Boston MA 02115-6110				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT  Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT  The overall aim of the study is to better understand glial metabolism within the context of RBT and AD and its potential findings in veterans. While the delay in the IRB approval has been frustrating, it has allowed for the resolution of a number of technical issues. The most significant finding in this report is the availability of 7 Tesla system for increased signal to noise ratio and greater spectral dispersion that allows for more accurate detection of dynamic glutamate changes after infusion of 13C-acetate that will be specific to glial cells. Combined with the previously described improvements utilizing denoising algorithms, there will be a significant improvement of data quality.					
15. SUBJECT TERMS Repetitive brain trauma, glial metabolism, glutamate, multinuclear spectroscopy, chronic traumatic encephalopathy, Alzheimer's disease, 13C acetate					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT  Unclassified	18. NUMBER OF PAGES  9	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT  Unclassified	b. ABSTRACT  Unclassified	c. THIS PAGE  Unclassified			19b. TELEPHONE NUMBER (include area code)

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## 1. INTRODUCTION:

Glutamate is a key compound in cellular metabolism with its most important role as a neurotransmitter with which the brain utilizes 80% of energy consumption to maintain this important cycle. Elevated levels of glutamate have been shown to be predictive of outcome in severe traumatic brain injury and our preliminary data from existing studies, have shown that glutamate remains elevated in the chronic stages of repetitive brain trauma (RBT) as well. Current methods of measure brain glutamate using spectroscopy is not specific to different cell types or the dynamic changes that undergo metabolism. We have developed a novel, non-invasive, quantitative method of measuring the dynamic rates of glutamate using  $^{13}\text{C}$ -labeled acetate, the primary fuel for glial cells, which can be tracked through the cerebral glutamate synthesis cycle using magnetic resonance spectroscopy. Our goal is to utilize infusion of  $^{13}\text{C}$ -labeled acetate in our existing cohort of retired NFL athletes with and without increased glutamate, subjects with Alzheimer's disease (AD), military veterans with a history of traumatic brain injury, and age-matched controls to measure the effect of repetitive brain trauma upon glutamate metabolism. Our hypothesis is that increased glutamate found in these players, will be reflected in up-regulation of glial pathways. The result of the study would be to identify the dysfunctional pathways that underlie glutamate excitotoxicity in sports-related brain trauma. These dysfunctions will provide precise targets for existing glutamate medications that are known to modulate specific pathways. Therefore we anticipate not only providing a better understanding of the metabolic mechanisms of sports-related head injury but also to provide data that will be useful for the development of much needed treatments for this devastating disease.

## 2. KEYWORDS:

Repetitive brain trauma, glial metabolism, glutamate, multinuclear spectroscopy, chronic traumatic encephalopathy, sports-related brain injury, military-related brain injury, Alzheimer's disease,  $^{13}\text{C}$  acetate

## 3. ACCOMPLISHMENTS:

### 3.1. Major Goals

Our overall aim will be to better understand glial metabolism within the context of RBT and AD and its potential findings in veterans. Specifically, we will:

Aim 1: Determine the mechanism (excitotoxicity?) that results in increased cerebral glutamate and glutamine (Glx) levels by comparing glial metabolic rates in NFL athletes with the highest levels of Glx and those with the lowest levels.

Aim 2: Determine the mechanism (neurodegeneration?) that results in decreased cerebral Glx levels by comparing glial metabolic rates in NFL athletes with the lowest levels of Glx and AD patients.

Aim 3: Identify the specific metabolic pathway that results in alternations of cerebral Glx levels in military and healthy controls as well as in comparison with NFL and AD subjects.

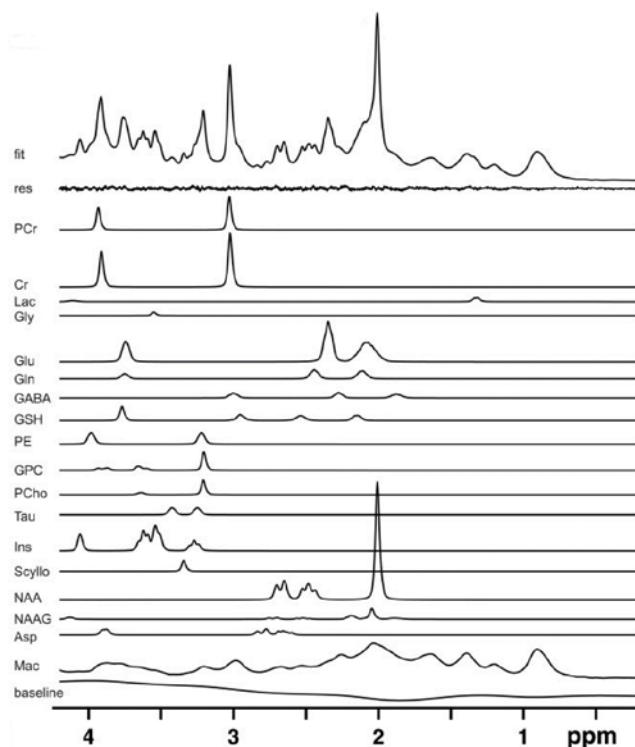
Aim 4: Correlate the glial and glutamate metabolic rates with additional measures obtained in the parent studies including of a) serum, CSF, and genetic biomarkers in the NFL subjects and b) neurocognitive

measures in all cohorts. The result of the study would be to identify the underlying physiological changes in glial metabolism in RBT such as neuroinflammation and glutamate excitotoxicity thus providing targets for much needed treatments as well as provide a safe, non-radioactive test to monitor these treatments.

### 3.2. Goal Accomplishments

Major Task 1 under Aim 1 is to submit local IRB protocol of amend existing study protocols. This has been extraordinarily delayed due to issues with hardware compliance of the  $^{13}\text{C}$  coil. Much thanks to the scientific review officer we were able to overcome this issue by allowing a change of protocol to utilize the new Siemens 7T TIM Terra scanner which was recently FDA approved and therefore faces no issues with compliancy. I am happy to state that we have now passed the MR safety requirements and the amendment to utilize the 7T system has been approved. The protocol has also passed MCA determination and the remaining item is Pharmaceutical review. We are confident that IRB approval will be obtained in the month of November. We have also completed FITBIR review of our protocol and have resolved the issues regarding the upload of ancillary data from our collaborators at Boston University.

While we recognize that we can not start this study with IRB approval, every effort has been made to prepare for the start of the study once IRB approval is obtained. We have therefore been focusing on optimization of the 7T spectroscopy protocol and have obtained preliminary data using funds provided by the our radiology department and the use of a protocol development IRB that allows for healthy subjects to be test. Our results, as expected, demonstrate an increased signal to noise ratio and spectral disperstion that will allow for the accurate detection of glutamate using proton spectroscopy (Figure 1). However, due to the increase in chemical shift, the localization of the voxel is misaligned using the conventional point-resolved spectroscopy (PRESS) and therefore we have been testing a stimulated echo acquisition method (STEAM) sequence which provides improved localization as well as the advantage of allowing for shorter echo times. As a result, glutamate, which has a very short T2 relaxation time, can take advantage of the 12 ms echo times that can be achieved with this sequence. We are still in the process of obtaining data however preliminary studies show that these technical improvements result in a much lower variance in the glutamate signal which will provide greater sensitivity for the sequentially acquired scans that will be used to monitor the glutamate levels during acetate infusion. In addition, we have completed the studies of the denoising methods and have developed model systems for determining the optimal denoising algorithm to be used for each dataset (see Section 3.4).



**Figure 1. Representative spectra acquired at 7 Tesla.** The top spectrum shows the complete spectrum and the individual spectral below it show the different metabolite resonances as a linear combination model. Note the high SNR of glutamate and its separation from other metabolites that would overlap at 3 Tesla.

### 3.3. Training and Professional Development

While this study does not have a component for training and professional development, Dr. Stern and I co-supervise and mentor Dr. Michael Alosco through an F32 Kirchstein Post-doctoral award. Dr. Alosco has now taken the place of John Jarnagin and will be assisting with the research study. Dr. Alosco brings with him significant background in neurovascular studies and as a result we have submitted and successfully obtained complimentary funding from the Alzheimer's Association to also include arterial spin labeling in this study. This will be a separate protocol offered to participants in the study but will not be mandatory and thus will not impact this study in any way.

### 3.4. Results Dissemination

In the previous progress reports, we described the development of denoising methods for the analysis of sequential MRS data as will be obtained in this study. This work has now been written up as a manuscript which was submitted for peer-review to Magnetic Resonance in Medicine, one of the leading journals in our field. While the findings remain the same, there are two other important results described in the manuscript in addition to the improvement in spectral quality after application of the denoising methods.

In order to test the optimal parameters for each of the denoising algorithms, we developed a method of creating pseudo-synthetic data that can be unbiased to the different algorithms. This is done by taking experimentally acquired data and combining it with the synthesized spectrum for more realistic analysis. This allows for optimization of data in different clinical scenarios (ie data acquired from young subjects versus older subjects) and provides a more customized approach for the denoising algorithms.

The second major development was to incorporate these testing methods into an open-source spectroscopy data analysis package which will allow any scientist to run these different algorithms on their own data. The software, called OpenMRS Lab is readily available on GitHub to any spectroscopy user and was initially supported by DoD funding from a previous study. This will allow these results to be readily disseminated to the scientific community for use in their own studies.

### 3.5. Next Reporting Period Plan

After local IRB approval is obtained, documentation will be sent to HRPO for approval. As soon as we receive HRPO approval, we will begin recruitment of subjects. We have subjects already lined up to participate in the study.

## 4. IMPACT:

### 4.1. Principle Discipline

Indirect detection of  $^{13}\text{C}$ -acetate using 7T proton spectroscopy has not been utilized before in other studies and therefore will provide highly novel and impactful results. As more and more sites move to higher field strengths, there has not been major developments in our field aside from the improved resolution of images. This method takes advantage of several innovative improvements and could potentially provide a new area of data acquisition that has not been fully explored. We are convinced that publications from this study will be eminently publishable in high impact journals given its technical novelty and important clinical implications.

## 4.2. Other Disciplines

While the technical improvements impact the radiological fields, the availability of a method to assess glial metabolism, specifically that of glutamate, will provide a non-invasive insight that will not only advance our understanding of glial changes in repetitive brain trauma and neurodegenerative diseases but will also provide potential targets for treatments for in this field that can impact disciplines of pharmaceutical research and ultimately for clinical treatment of long-term impacts of repetitive head trauma.

## 4.3. Technology Transfer

The availability of the denoising methods through open-source software will allow for the ready transfer of the technology developed in this study to the scientific community. All too often the scientific community has difficulty in reproducing methods that are developed “in-house” as the details are often not available. With an open-source approach, much like the aim for FITBIR, these methods will be available for the whole of the scientific community and could provide potential benefits both in our field and other disciplines.

## Society

The publication of the denoising methods will provide advances in the field of research that can potentially impact society through improved diagnosis and detection of disease.

## 5. CHANGES/PROBLEMS:

### 5.1. Changes in approach

As described earlier, there have been two major changes to our approach: 1) changing the protocol to utilize proton spectroscopy instead of  $^{13}\text{C}$  spectroscopy. The advantage of this approach is that standard FDA-approved sequences and coils are used instead of research sequences and coils. Instead of directly detecting  $^{13}\text{C}$  signal, this method takes advantage of the fact that incorporation of the  $^{13}\text{C}$ -labeled acetate into glutamate results in heteronuclear coupling that reduces the proton glutamate signal. This reduction in signal can be tracked, to provide the rate of metabolism of  $^{13}\text{C}$  glutamate and glutamine as described in our original proposal. The major advantage of this method is that it offers much greater signal without the use of specialized equipment. This will allow for rapid dissemination of this method to other sites that would likely not have  $^{13}\text{C}$  multinuclear-capable systems and coils. The disadvantage to this method is that the specificity of the glutamate and glutamine are impacted by the overlap between the two resonances. Thus the second change 2) changing from 3 Tesla to 7 Tesla. The advantage of 7 Tesla is not only improved signal-to-noise ratio but also increased spectral dispersion due to the increase in the dynamic range of the signal. This increases spectral resolution such that glutamate and glutamine can be readily separated and observed. This is in addition to the gains that will be obtained from the improved post-processing methods that we described above.

## 5.2. Actual or anticipated problems

The delay of the IRB approval has been a problem however we anticipate that this will be resolved within the next month and recruitment can begin immediately after IRB approval. In anticipation, we have prepared and tested the protocol and already have subjects identified to participate in the study.

## 5.3. Impact on expenditures

Due to the delay of the start of the study, we have significantly reduced spending on the grant so that a no-cost extension can be carried out next year. There will no impact on the budget to utilize the 7 Tesla system as the costs are the same as the 3 Tesla system.

## 5.4. Significant changes in use or care of human subjects

None.

# 6. PRODUCTS:

## 6.1. Publications, conference papers, and presentations

Rowland BC, Sreepada L, Lin AP. A comparison of denoising methods in dynamic MRS using pseudo-synthetic data. Magn Reson Med (submitted).

# 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Alexander Lin
Project Role:	Principal Investigator
Research Identifier:	<a href="https://orcid.org/0000-0001-8236-880X">orcid.org/0000-0001-8236-880X</a>
Nearest Person Month Worked	4
Contribution to Project	Dr. Lin has been addressing the concerns of the IRB and MR safety committees by conducting tests of the 13C coil for safety purposes. The results of the tests have been sent to the committee for evaluation.

Name:	Huijin 'Vicky' Liao
Project Role:	Study Coordinator
Research Identifier:	<a href="https://www.linkedin.com/in/huijun-vicky-liao-3b682451">https://www.linkedin.com/in/huijun-vicky-liao-3b682451</a>
Nearest Person Month Worked	4
Contribution to Project	Ms. Liao has assisted Dr. Lin with the acquisition of the 7T spectroscopy data and assisted with the submission of the IRB documentation.

Name:	Ben Rowland
Project Role:	Postdoc
Research Identifier:	n/a
Nearest Person Month Worked	3
Contribution to Project	Dr. Rowland has been preparing for the start of the project by improving the software required for the data analysis by developing six different denoising methods.



Name:	Robert Stern
Project Role:	Subaward PI
Research Identifier:	orcid.org/0000-0002-5008-077X
Nearest Person Month Worked	1
Contribution to Project	Dr. Stern has assisted with the recruitment process as described above.

Name:	Michael Alosco
Project Role:	Subaward Study Coordinator
Research Identifier:	n/a
Nearest Person Month Worked	2
Contribution to Project	Dr. Alosco has been in contact with both NFL and AD participants through his own work at BU and will assist with recruitment of subjects for this study.

### 7.1. Change in Personnel

Nothing to Report.

### 7.2. Other Organizations

Organization Name: Boston University School of Medicine

Location of Organization: Boston, Massachusetts

Partner's contribution to the project:

- Collaboration: Drs. Robert Stern and Michael Alosco are our collaborators and are responsible for recruitment of subjects, acquisition of neuropsych results, and upload of that data into FITBIR.

## 8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report.

## 9. APPENDICES:

None.